PS 1181 ELUCIDATION OF FACTORS DETERMINING CARBON NANOTUBES' ABILITY TO PENETRATE ALVEOLAR EPITHELIAL BARRIER AND INTERACT WITH LUNG FIBROBLASTS *IN VITRO*.

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Nanomaterials possess unique physicochemical and biological properties but can also exhibit different adverse reactions if inhaled. Our previous *in vivo* study showed upon alveolar deposition, dispersed single-walled carbon nanotubes (DSWCNT) rapidly enter interstitial area (1 day post-exposure) and induce interstitial fibrotic response as early as 1 week post-exposure. Direct stimulation of cultured lung fibroblasts, a major interstitial cell, by DSWCNT was shown to enhance proliferation and collagen production, a hallmark of lung fibrosis. Furthermore, penetration of DSWCNT through lung epithelial barrier into interstitium could be a key event of DSWCNT-induced interstitial fibrosis. To investigate this alveolar epithelial barrier, an experimental model was developed using immortalized human lung epithelial cell line (ATCC, Manassas, VA). Epithelial cells were cultured on the apical surface of Transwell® microporous membrane and exposed to non-dispersed SWCNT and DSWCNT. Samples from the apical compartment, cell monolayer, and basolateral compartment were collected at various times and analyzed for CNT penetrability. Electron microscopy and CytoViva hyperspectral imaging were used to aid characterization of the penetration pathway (paracellular vs. transcellular) of nanoparticles across alveolar epithelial membrane. The effect of CNT dispersion status on penetration rate was also assessed. Our data suggest CNT penetrated through epithelial cells on apical side and translocated to the other side of the Transwell membrane and the amount of CNT transferred, measured by hyperspectral imaging, was sufficient to induce fibroblast proliferation and collagen production based on previous data. The Transwell system is a suitable model for studying translocation of CNT across epithelial layer and aids in mechanistic studies of CNT- induced interstitial lung fibrosis.

PS 1182 *IN VITRO* ASSESSMENT OF POTENTIAL TUMORGENICITY OF CHRONIC SWCNT AND MWCNT EXPOSURE TO LUNG EPITHELIUM.

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Carbon nanotube use in multiple consumer and industrial products has resulted in increased concern for long-term risks to human health. Recent *in vivo* studies suggest that inhaled carbon nanotubes induce interstitial fibrosis and persist in exposed lung tissues. No clear evidence or experimental model exists to assess whether long-term pulmonary exposures of CNT to lung tissue results in transformed cells expressing a tumorigenic phenotype. This study tested the hypothesis that chronic exposure to dispersed carbon nanotubes induces neoplastic transformation in lung epithelial cells *in vitro.* Small airway lung epithelial cells were exposed for 25 weeks to either dispersed single wall (D-SWCNT) or multi-wall (D-MWCNT) carbon nanotubes at non-cytotoxic levels (0.02 μg/cm^2). Dispersed ultra fine carbon black (D-CB) and asbestsos (ABS) served as negative and positive controls. Following exposure each treatment was evaluated for tumorigenic phenotypes using cell proliferation, cell invasion and colony formation assays in untreated media. D-SWCNT exhibited a significant 1.5-fold increase in cell proliferation compared to passage control cells while D-MWCNT and D-CB showed a modest increase. In addition, D-SWCNT cells displayed significantly greater invasive potential than D-MWCNT, ABS and passage control. Lastly, a tumor formation assay resulted in D-SWCNT cells possessing a significant 5-fold increase in the number of colonies formed above controls while D-MWCNT and D-CB exhibited only a 1.5-fold increase. Our chronic, low dose *in vitro* exposure model suggested that D-SWCNT exposure caused neoplastic transformation resulting in a tumorigenic phenotype while D-MWCNT exposure displayed less malignant potential. In conclusion, use of *in vitro* screening methods, along with comparable *in vivo* data, can assist in investigation of tumorigenic risks associated with carbon nanotube and other nanomaterial exposures.

PS 1183 ASSESSMENT OF FIBROGENIC BIOMARKERS INDUCED BY MULTI WALL CARBON NANOTUBES.

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Multi Wall Carbon Nanotubes (MWCNT), a graphene based nanoparticle, possess unique physiochemical properties. Considered as a technological breakthrough, production of MWCNT is rapidly increasing worldwide but toxicity profile of the nanomaterials is not clearly understood. Among the adverse effects, CNT have been shown to induce (the development of unusual) interstitial lung fibrosis at physiologically relevant exposure (10μg/mouse); however, the underlying mechanism is not fully known. In this present study, we investigated important MWCNT-induced fibrogenic mediators using cultured lung cell systems. Human bronchial epithelial (BEAS-2B) cells, alveolar epithelial (A549) cells, and lung fibroblast (CRL1490) cells were treated with MWCNT. Viability of MWCNT-exposed cells was determined by cell counting and WST-1 assay. Fibrogenic mediators, including Fibroblast Growth Factor-2, Vascular Growth Factor, Transforming Growth Factor β1 (TGF-β1), and Platelet Derived Growth Factor-A, were analyzed using Western Blots and end point ELISA. Our results show that 1) MWCNT decreased cell viability of epithelial cells in a dose and time dependent manner, 2) MWCNT exposure induced secretion of fibrogenic mediators from lung epithelial cells and fibroblasts at physiologically relevant concentrations of 0.02 to 0.6 μg/cm^2 and 3) MWCNT directly induced collagen production from lung fibroblasts. In conclusion, MWCNT induced fibrogenic mediators in cultured human lung epithelial cells and stimulated collagen production from fibroblasts. These data are consistent with *in vivo* observations. Therefore, the *in-vitro* cell culture systems can be used for mechanistic studies and screening tests for MWCNT and similar fibrogenic nanoparticles.

PS 1184 DIFFERENTIAL EFFECTS OF SINGLE-WALLED CARBON NANOTUBES ON HUMAN HEPATIC, RENAL, AND COLORECTAL CARCINOMA CELL LINES.

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Carbon nanotubes (CNTs) are currently one of the promising materials for the development of nano-technologies. However, CNT toxicity is a major concern. Many in vitro studies have assessed the cytotoxicity of CNTs, but the results differ according to the cell lines and catalysts used for synthesizing CNTs. We have previously reported the differential effects of single-walled CNTs (SWCNTs) on human lung carcinoma A549 and human head and neck carcinoma FaDu cells (SOT 2010 annual meeting). The present study aimed to clarify the cytotoxic effects of SWCNTs on HepG2, ACHN, and Caco-2 cells derived from the human liver, kidney, and colon, respectively, because SWCNTs are expected to be used as drug and gene carriers in medical fields. The SWCNTs used in this study were manufactured using 2 types of arc electrical discharge method, with Ni and Y (SO-SWCNTs) and Fe (FH-P-SWCNTs) as catalysts. Cell viability was evaluated on the basis of the ATP content and metabolic capacity of the cell. SWCNTs were exposed to the cells at concentrations of up to 1.0 mg/ml. On 24-h exposure of 1.0 mg/ml SO-SWCNTs to HepG2, ACHN, and Caco-2 cells, the ATP content of these cells decreased to 91%, 87%, and 90% of the ATP content of untreated cells, respectively. Under identical conditions, HepG2, ACHN, and Caco-2 cells exposed to FH-P-SWCNTs showed similar results (decrease in ATP content to 91%, 97%, and 93%, respectively). However, the metabolic capacity of SO-SWCNT-exposed cells was slightly higher than that of FH-P-SWCNT-exposed cells, that is, 63%, 88%, and 69% vs. 50%, 93%, and 48% for HepG2, ACHN, and Caco-2 cells, respectively. These results suggest that exposure to SWCNTs has a greater effect on metabolic activity than on ATP content of these cells and that ACHN cells are most resistant to SWCNT exposure. The present study clarified that the effects of SWCNTs on cell viability differed depending on the type of cell, SWCNT, and analytical method used to assess cell viability.

PS 1185 MULTI-WALL CARBON NANOTUBE (MWCNT)-INDUCED GENE EXPRESSION IN THE MOUSE LUNG: IMPLICATION OF CARCINOGENESIS RISK.

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MWCNT's fibrous-like shape and durability suggest they may pose a similar carcinogenic effect on humans as asbestos. Therefore, this study sought to investigate how previously identified lung cancer prognostic biomarkers and the related cancer signaling pathways are affected in the mouse lung following in vivo pharyngeal aspiration of MWCNT. A total of 63 identified lung cancer prognostic biomarker genes were analyzed using quantitative PCR assays in the mouse lung exposed by aspiration to 0, 10, 20, 40, 80 μg of MWCNT at 7-days and 56-days post-exposure. At 7- and 56-days post-exposures, a set of 7 genes and a set of 11 genes showed significant differential expression in the mouse lungs exposed to MWCNT vs. unexposed control groups, respectively. These significant genes could clearly separate

the control group from the treated group over the time series in hierarchical gene clustering analysis. Furthermore, 4 genes from these two sets of significant genes, Ccdc99, Msx2, Nos2 and Wif1, showed significant mRNA expression perturbations at both time points. It was also found that the expression changes of these 4 overlapped genes at 7-days post-exposure were attenuated at 56-days post-exposure. The results of MWCNT exposure-induced gene expression changes reveal the characteristics of carcinogenesis and may indicate the association of MWCNT exposure with lung cancer progression. These results also indicate that MWCNT exposure may induce the alteration of several key carcinogenesis-related signaling transduction pathways. Taken together, the results obtained from this study indicate the potential lung carcinogenetic effects of MWCNT exposure in humans and could potentially be used for the medical surveillance for MWCNT workers.

PS 1186 MULTI-WALLED CARBON NANOTUBE INSTILLATION IN C57BL/6 MICE INDUCES CHANGES IN PULMONARY FUNCTION.

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As the production and use of nanomaterials increases, the potential for human exposure also increases thereby placing greater emphasis on potential health hazards. Multi-walled carbon nanotubes (MWCNT) in particular, are widely used for their versatility in many disciplines due to their unique physical and chemical properties. The purpose of this study was to examine the long-term pulmonary inflammatory and fibrotic effects of MWCNT at 7, 30, 60 and 90 days post-exposure. Inflammatory and fibrotic responses in lungs of C57BL/6 mice following MWCNT instillation were assessed by cytokine expression, bronchoalveolar lavage cell counts, collagen content, pulmonary function testing and histological assessment. Mice instilled with MWCNT (100 μg) exhibited increased expression of pro-inflammatory and pro-fibrotic cytokines, which were associated with increased infiltration of macrophages, neutrophils, eosinophils and lymphocytes up to 60 days post-exposure. In addition, pulmonary function testing demonstrated an increase in the area within the pressure-volume loops (PV loops) for MWCNT treated mice compared to vehicle control indicating a change in lung hysteresis. These findings corroborate histological data exhibiting increased granuloma formation and development of fibrosis up to 90 days associated with increased collagen content in lung tissue of C57BL/6 mice instilled with MWCNT. These data indicate that exposure to MWCNT results in adverse pulmonary effects, including increased pulmonary inflammation, fibrosis and compromised lung function. This work supported by NIH RO1 ES019311 (JMB) and ES016246 (CJW).

PS 1187 CARBON NANOTUBES INDUCE APOPTOSIS RESISTANCE THROUGH FLICE-INHIBITORY PROTEIN.

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Our studies have shown that chronic exposure to single-walled carbon nanotubes (SWCNT) induces apoptosis resistance and malignant transformation of human lung epithelial cells. Since resistance to apoptosis is a foundation of neoplastic evolution and selection of malignant transformed phenotype, we investigated the apoptosis pathway underlying the resistance its mechanisms to aid the understanding of SWCNT-induced carcinogenesis. As compared to passage-matched control cells, SWCNT-transformed BEAS-2B cells exhibited resistance to apoptosis induced by death ligands, e.g., tumor necrosis factor-a and Fas ligand, but not by inducers of mitochondria-mediated apoptosis, e.g., antimycin A and cisplatin, suggesting death receptor pathway as the primary pathway of defective apoptosis in SWCNT-transformed cells. The results were confirmed using caspase specific inhibitors and caspase activity assays. DNA microarray and Western blot analyses of key apoptosis-regulatory proteins in the transformed cells revealed FLICE-inhibitory protein (FLIP) as an important target of regulation by SWCNT. Overexpression of FLIP increased apoptosis resistance of the cells, whereas RNAi knockdown of FLIP reversed the apoptosis resistance of cells in response to death ligands. Together, our study demonstrated a novel mechanism of apoptosis resistance induced by chronic exposure to SWCNT in human lung epithelial cells and identified FLIP as a key regulator of apoptosis avoidance that contributes to the development of malignant transformed phenotype. [This work is supported by the NIH grant R01-HL095579]

PS 1188 POTENTIAL CARCINOGENICITY OF CARBON NANOTUBES.

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Carbon nanotubes (CNT) have increasing been used for wide applications with a potential for human exposure. Concerns about the potential carcinogenicity of CNT have been raised since CNT exhibit a bio-persistence and a fibrous morphology similar to asbestos which is a known carcinogen. However, there is neither clear knowledge nor a practical method to assess this potential. In this study, we developed an in vitro chronic exposure model combined with in vivo xenograft model to address these needs. Non-tumorigenic human lung epithelial BEAS-2B cells were continuously exposed to a sub-cytotoxic concentration (0.04 ug/ml or 0.02 ug/cm2 exposed area) of single-walled CNT (SWCNT) in culture. Phenotypic changes were observed in SWCNT-treated cells 20 weeks post-exposure such as formation of cell mounds and accelerated cell growth. SWCNT-treated cells were subsequently analyzed for malignant properties including colony formation, cell migration and invasive properties. Significant positive results were observed from SWCNT-treated cells in all above studies compared to passage-matched control cells. In vivo tumorigenesis study was performed by subcutaneously injecting the transformed cells into nude mice. Consistent with the in vitro cell transformation results, the in vivo results showed large tumor formation at the injection site in mice receiving SWCNT-transformed cells, whereas mice receiving control cells showed no tumor formation. These studies indicate that long-term/low dose exposure of human lung epithelial cells to SWCNT induced malignant transformation of the cells which induced tumor formation in vivo. These results suggest a potential carcinogenic effect of SWCNT. The described cell model system could potentially be used as a predictive model for carcinogenicity testing of nanomaterials. [This work was supported by the NIH Grant R01-HL076340]

PS 1189 PULMONARY EXPOSURE TO CARBONACEOUS NANOPARTICLES AFFECTS LOCAL AND SYSTEMIC IMMUNITY.

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Numerous studies have focused on the toxicity associated with nanoparticle (NP) exposure. The results of studies on rodents have demonstrated that NP are capable of inducing inflammation, granulomas, fibrosis, and mutagenicity found in the lungs. However, immunologic effects of inhaled nanoparticles remain largely unexplored. In the current study, we evaluated the inflammatory response in the lung and systemic immune effects induced by pulmonary exposure to 40-120 μg/mouse of pristine (C_{60}) or functionalized (C_{60}-TRIS) fullerenes or single-walled carbon nanotubes (SWCNT). We demonstrated that pharyngeal aspiration of the studied NP caused inflammation and pulmonary damage as evidenced by accumulation of PMNs, changes in lung permeability and cell damage. In addition, NP stimulated release of pro-inflammatory and regulatory cytokines in the lung. Further, local inflammatory response was translated into suppressed systemic immunity as evidenced by 25% decrease in proliferation of splenic T cells stimulated by allogeneic dendritic cells (DC). To investigate possible mechanisms of compromised systemic immunity, we evaluated the ability of NP to directly affect stimulatory/polarizing activities of conventional DC (cDC) towards T cells *in vitro*. Exposure of cultured cDC to NP resulted in an impaired ability to stimulate T cells in an allogeneic MLR assay (up to 4-fold suppression). This effect was due to neither altered expression of CD80, CD86 or MHCII on DC as exhibited by DC phenotyping, nor by increased production of IL-10. Thus, mechanisms of altered systemic immunity in treated mice might be, at least in part, due to the direct effects of NP on cDC. Overall, our data suggest that NP affect and trigger both local and systemic immune responses in mice.
Supported by NIOSH OH008282, NORA 0HELD015, and EC-FP-7-NANOMMUNE-214281.

PS 1190 PULMONARY RESPONSE OF CIGARETTE SMOKE-EXPOSED MICE TO CARBON NANOPARTICLES.

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Concurrent or sequential exposures to more than one air pollutant can potentially affect pulmonary toxicity. Carbon nanoparticles when instilled into the lungs of mice induce an acute pulmonary response. To determine if such a response is influ-

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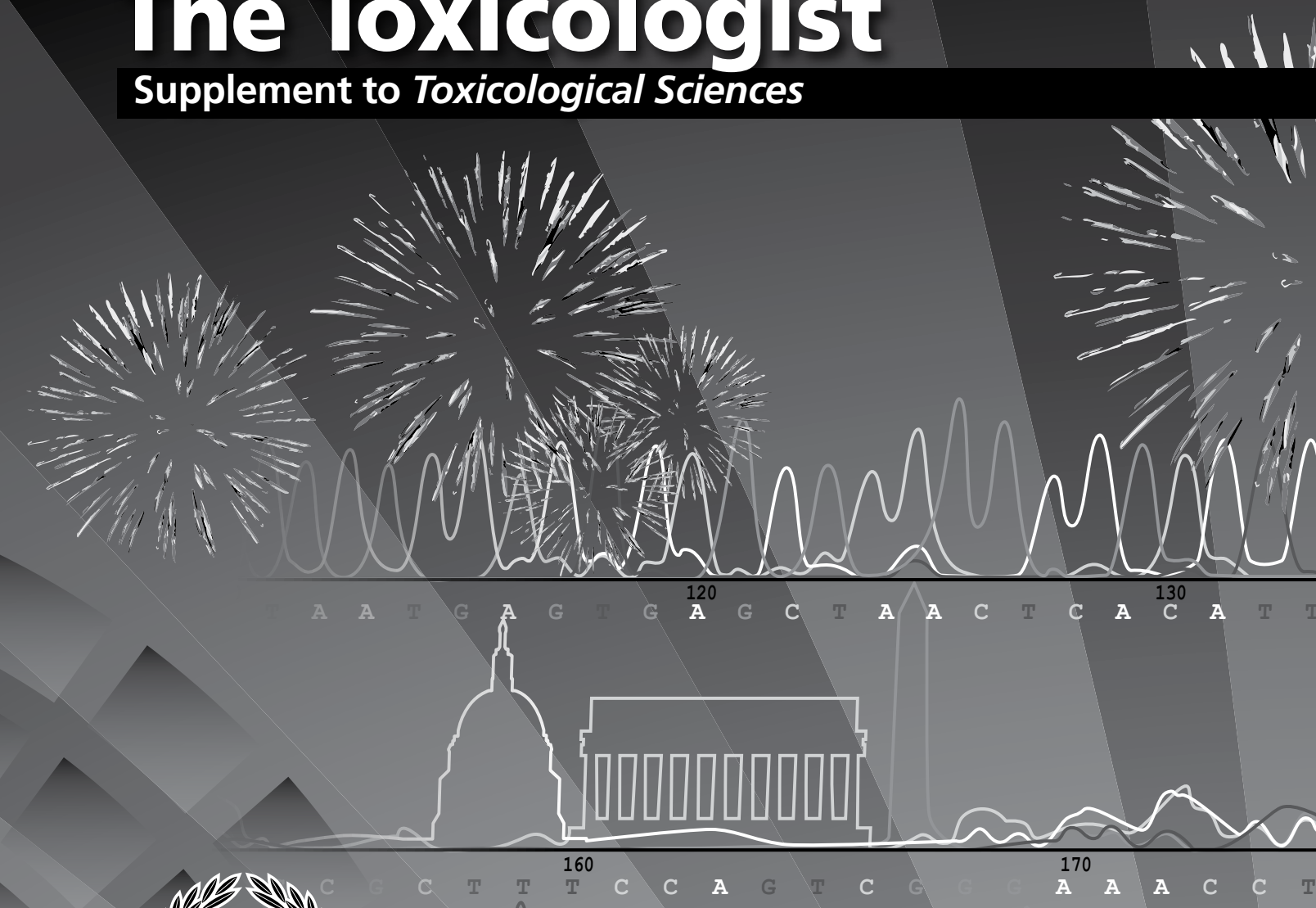

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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